Abstract

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Title of diploma thesis: Preparation of mammalian vectors encoding selected microsomal SDR enzymes

Microsomal short-chain dehydrogenases/reductases DHRS1, DHRS7 and HSD11b1 (SDR19C1, SDR34C1, SDR26C1) are membrane-bound NAD(P)H-dependent enzymes metabolizing a broad spectrum of carbonyl-bearing substrates. These enzymes from the superfamily of short-chain dehydrogenases/reductases mediate metabolism of endogenous compounds, such as glucocorticoids, retinoids, sfingolipids as well as various xenobiotics. Apart from their biological roles, they participate in the etiology of severe diseases (e.g cancer, Alzheimer disease, obesity etc.). Knowledge of inhibitory and substrate affinity may lead to better understanding of the functions of these enzymes in organism and to the development of new therapeutic approaches. The goal of the present work was to prepare mammalian vectors encoding DHRS1, DHRS7 and HSD11b1.

Primer design and cDNA amplification were among the first steps. Primers beared the sequence recognized by restriction endonucleases which was concomitantly present in the multicloning site of the pCI plasmid. Such primer design allowed for generation of cohesive ends and consequent easy joining of insert with plasmid by ligation. Prepared vectors were transformed into *Escherichia coli* HB101 cells. Fruitfullness of insert implementation was evaluated by electrophoresis and control and back restriction in plasmids isolated from chosen colonies. Plasmids that seemed to have successfully encloned insert were amplified, isolated and purified in midi format and verified by sequencing analysis.

Mamalian pCI vectors encoding DHRS1, DHRS7 and HSD11b1 were succesfully prepared. These vectors are going to be employed for transfections in future studies focused on the characterization of inhibitory and substrate affinities of selected enzymes.